Experimental infections with a honeybee virus induce fitness costs in ant colonies

Lai Ka Lo[1, 2], Ashmita Baruah[4], Robert J. Paxton[2, 3], Yuko Ulrich[1, 2]

Affiliations:

[1] Lise Meitner Research Group Social Behaviour, Max Planck Institute for Chemical Ecology, Jena, Germany

[2] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Leipzig, Germany

[3] General Zoology, Institute for Biology, Martin Luther University Halle-Wittenberg, Halle (Saale), Germany

[4] Indian Institute of Science Education and Research, Pune, India

Corresponding Authors:

Yuko Ulrich, yulrich@ice.mpg.de; Lai Ka Lo, llo@ice.mpg.de

ORCID:

Lai Ka Lo	0000-0002-8289-9229
Robert J. Paxton	0000-0003-2517-1351
Yuko Ulrich	0000-0001-9380-7863

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Abstract:

Acute Bee Paralysis Virus (ABPV) is a key driver of honey bee colony losses that has been increasingly reported in non-bee hosts. Ants have long been hypothesised to act as viral reservoirs, but most evidence comes from field surveys, and experimental tests are still scarce. Here, we combined survival and transmission assays, viral load quantification, and viral replication assays following experimental inoculations to test whether the clonal raider ant *Ooceraea biroi* can harbour and transmit ABPV within its colonies. ABPV-injected immatures and adults showed delayed development and elevated mortality, respectively. Viral replication assays suggested these fitness costs were caused by host responses (e.g., immunopathology) rather than viral replication. Viral particles persisted for days in inoculated ants and high viral loads were detected in untreated colony members after 1–3 days of cohabitation with ABPV-injected pupae or adults, indicating rapid viral spread. These results show that ants can acquire ABPV, incur fitness costs, and pass the virus within their colonies, suggesting that

they may act as incidental viral reservoirs. By maintaining and disseminating honey bee viruses, even without supporting replication, ants may contribute to their environmental persistence and spillover across species.

1. Introduction

Honey bees are vital pollinators for ecosystems and agriculture, yet highly vulnerable to human activities and environmental changes. Over the past two decades, substantial winter colony losses of western honeybees, *Apis mellifera*, have been reported worldwide (Neumann & Carreck, 2010). Several iflaviruses and dicistroviruses have emerged as major drivers of colony losses in both managed and wild populations (Carreck et al., 2010; Genersch & Aubert, 2010; McMenamin & Genersch, 2015; Neumann & Carreck, 2010; Yañez et al., 2020). While these viruses can persist covertly at low titres without causing apparent symptoms, the introduction of the ectoparasitic mite *Varroa destructor* (henceforth: varroa) has facilitated viral spread and turned asymptomatic infections into lethal diseases (Wiegers, 2021) by injecting viruses into the bee haemolymph (Ryabov et al., 2014).

Acute Bee Paralysis Virus (ABPV), a +ssRNA virus in the family *Dicistroviridae* (Benjeddou et al., 2001; de Miranda et al., 2010), has been detected repeatedly in collapsing and *Varroa*-infested colonies and is considered a significant driver of honey bee mortality (Ball, 1985; Ball & Allen, 1988; Berthoud et al., 2010; Cox-Foster et al., 2007; de Miranda et al., 2010; Žvokelj et al., 2020). It occurs across all host life stages and castes, with pupae being especially permissive to viral replication (Azzami et al., 2012; Bailey & Milne, 1969; de Miranda et al., 2010; Žvokelj et al., 2020). Experimental infections have shown that the injection of fewer than 100 viral particles into pupae or adults can induce tremors, paralysis, and death within days (Bailey et al., 1963). ABPV has been detected outside honeybees (Doublet et al., 2025; Gusachenko et al., 2020; McMahon et al., 2015; Pascall et al., 2021) and is considered a multi-host virus, with cross-species transmission facilitated by shared floral resources and nesting sites (Yañez et al., 2020).

In recent years, ants have been proposed as viral reservoirs for ABPV based on repeated field detections (Celle et al., 2008; Gruber et al., 2017; Levitt et al., 2013; Lin et al., 2020; Payne et al., 2020; Rivas Fontan et al., 2025; Sébastien et al., 2015). With an estimated 20 quadrillion individuals and a biomass exceeding that of all wild birds and mammals, ants are globally dominant insects (Parker & Kronauer, 2021; Schultheiss et al., 2022) whose foraging and nesting ranges overlap with those of many taxa, making them potential pathogen vectors (Dobelmann et al., 2023; Tiritelli et al., 2025). ABPV has been reported in ants and honey bees from ant-infested hives, suggesting ants as viral vectors within apiaries (Rivas Fontan et al., 2025). Yet, most studies remain observational, and whether the virus can persist, transmit, and replicate in ant, is largely unknown. Experimental work is limited to one study, which

showed that oral exposure to ABPV impaired locomotion and colony growth in *Lasius niger* (Schläppi et al., 2020).

Here, we investigate ABPV infections in the clonal raider ant *Ooceraea biroi*, a powerful model for studying disease dynamics under controlled laboratory conditions. Workers in *O. biroi* colonies reproduce clonally and synchronously (Ravary et al., 2006), allowing precise experimental control over genotype and age at the individual and colony levels. In addition, the predatory lifestyle of *O. biroi*, whose diet includes ants and other insects (Kronauer, 2009), makes them prone to pathogen spillover. To investigate the consequences of ABPV exposure in this system, we measured the effects of controlled inoculations on host survival and assessed the ability of the virus to replicate within hosts and transmit within colonies.

2. Materials & Methods

(a) Ant rearing

All ants were sourced from stock colonies of genetic lineage B (Kronauer et al., 2012), and maintained at 28 °C in airtight plastic containers (8 x 12 x 5 cm) with a humidified plaster of Paris floor (Knauf) before experiments. The nests were regularly cleaned, re-humidified, and fed with sterilized, frozen ant brood (*Tetramorium bicarinatum* and *Oecophylla* sp.) and houseflies. For each experiment, all ants were clonally related.

(b) Infection assay in pupae and adults

(i) Preparation of viral inoculum

The ABPV inoculum was prepared as described by Tehel et al. (2019). Briefly, 1 µL of homogenate from a heavily ABPV-infected *A. mellifera* worker was injected into white-eyed pupae obtained from virus-free colonies. The injected pupae were incubated for three to five days to allow viral replication and systemic infection, after which they were homogenised in 0.5 M cold potassium phosphate buffer (PPB, pH 8.0). The resulting homogenates were screened using RT-qPCR to confirm the absence of other common bee viruses, such as deformed wing virus (DWV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), Israeli acute paralysis virus (IAPV), sacbrood virus (SBV), and slow bee paralysis virus (SBPV). Only homogenates confirmed to contain ABPV exclusively were used in the experiments. Control inoculum was prepared from uninjected white-eyed pupae processed in the same way.

(ii) Inoculation via microinjection

10-day old ant pupae were placed on glass slides and injected with viral inoculum (ABPV at 10^5 genome equivalents, GEs) laterally at the posterior abdomen using a FemtoJet microinjector (Pi = 200, Pc = 30; Eppendorf AG, Hamburg, Germany) and borosilicate glass capillaries (8.89 cm length, 0.530 mm ± 25 µm inner diameter, 1.14 mm outer diameter; World Precision Instruments). Inoculum was injected until the pupal body visibly stretched. Control pupae were injected with the control inoculum following the same procedure. Following inoculation, eight pupae of either treatment were placed together with six paint-marked, uninjected cohabiting adults in plastic cups (diameter 4.5 cm) with a humidified plaster of Paris floor. For each treatment (control and ABPV-injected) and each sampling time point (0, 3, and 5 days post-inoculation, d.p.i.), four replicate colonies were established. At time point 0, pupae were flash-frozen immediately after injection. At later time points (3 and 5 d.p.i.). Both pupae and cohabiting adults were frozen to assess viral transmission. Pupae survival and development were assessed in the colonies sampled 5 d.p.i. (four control and four infected

127 colonies). Pupae that disappeared, turned dark, or were partly cannibalized were classified as
128 dead.
129
130 Adult ants (mixed age) were immobilised by placing them in slits cut in pencil erasers (Läufer
131 Plast 0120) and injected with 10 nL of viral inoculum (ABPV 5×10^4 GEs) or control inoculum
132 at a rate of 5 nL/s under the largest abdominal tergite using a NanoFil 36 GA bevelled needle
133 connected to a NanoFil syringe and an electronic microinjection pump (UMP3 pump with
134 Micro2T SMARTouch controller, World Precision Instruments, USA). Ten injected adults from
135 either treatment were housed with five paint-marked, uninjected cohabiting adults in plastic
136 cups (diameter 4.5 cm) with a humidified plaster of Paris floor. For each treatment (control and
137 ABPV-injected) and each sampling time point (0, 1, 3, and 5 d.p.i.), four replicate colonies
138 were established. At time point 0, focal ants were flash-frozen immediately after the injection.
139 At later time points (1, 3, and 5 d.p.i.), both focal ants and cohabiting adults were flash-frozen.
140 The colonies sampled 5 d.p.i. were monitored for survival over the full period before sampling.
141
142 **(c) Viral load quantification**
143 For each colony, five injected adults or three injected pupae were flash-frozen in liquid nitrogen
144 and stored at -80 °C. In the ABPV-injected groups, five cohabiting adults were also sampled.
145 Total RNA was extracted from pooled, whole-body samples using a combined Trizol/phenol-
146 chloroform-column protocol (Libbrecht et al., 2016) and eluted in 20 µL of nuclease-free water.
147 RNA concentration and purity were assessed using a BioPhotometer D30 (Eppendorf,
148 Germany).
149
150 Viral load quantification was performed according to a protocol modified from Tehel et al.
151 (2019). For cDNA synthesis, 200 ng of total RNA was reverse-transcribed using oligo(dT)18
152 primers (Thermo Scientific) and reverse transcriptase (M-MLV and Revertase, Promega,
153 Mannheim, Germany) according to the manufacturer's instructions in a Veriti Thermal Cycler
154 (Applied Biosystems). The resulting cDNA was diluted 1:3 and stored at -20 °C. qPCR was
155 performed using the KAPA SYBR FAST Universal kit (Kapa Biosystems, Sigma-Aldrich) on a
156 LightCycler 480 (Roche), with two technical replicates per sample, and the following thermal
157 cycle: three min at 95 °C; 40 cycles of 10 s at 95 °C, 30 s at 57 °C, 20 s at 72 °C; final
158 elongation for 1 min at 95 °C; melt curve analysis was performed from 55 °C to 95 °C with 0.5
159 °C per second increments to check for amplification specificity. Forward primer (5′-
160 CATGGCTCAAGACACTTCATCG-3′) and reverse primer (5′-CCAGCAATGACCTCAATGTG
161 G-3′) were used to amplify a 105 bp fragment specific to ABPV capsid protein gene, were
162 designed with Primer-BLAST (Ye et al., 2012). Each plate included negative controls (control
163 inoculum and no-template control) and a positive control (viral inoculum). Samples with Ct

164 values >35 or with high variation between technical duplicates (Ct standard deviation >0.5)
165 were re-assayed, except for negative controls and control inoculum, with actin (host reference
166 gene) amplified using primers Ob-actinF (5'-GCCGATCGTATGCAGAAGGA-3') and Ob-
167 actinR (5'-ACCCTTGGCGATGTGTCTTT-3') designed with Primer-BLAST. To quantify the
168 number of viral GEs in each sample, external DNA standards were generated from the viral
169 inoculum using primers ABPV-F6531 (5′-TCATACCTGCCGATCAAG-3′) (Locke et al., 2012)
170 and ABPV-R6774 (5'- CCAGCAATGACCTCAATGTGG-3'). The PCR product size (244 bp)
171 was verified by electrophoresis on a 2% agarose gel, and the corresponding band was excised
172 and purified using the NucleoSpin Gel and PCR Purification Kit (Macherey-Nagel, Düren,
173 Germany) according to the manufacturer's instructions. The purified PCR product was
174 quantified using a spectrophotometre and used to generate ten-fold serial dilutions. Viral loads
175 were expressed as $\log_{10}$ GEs per individual ant by dividing total GEs by the number of
176 individuals in each sample.
177

178 **(d) Negative-sense strand detection**

179 In a separate experiment, three-week-old adult ants were injected with 10 nL of viral inoculum
180 (ABPV 10^4 GEs), control inoculum, or left uninjected; five focal adults from each treatment
181 were housed with five paint-marked, uninjected cohabiting adults, as described above. A lower
182 dose was chosen to increase survival and extend the observation period. For each treatment
183 and time point (0, 6, 12, 24, and 48 hours post-injection, h.p.i.), four replicate colonies were
184 established. At each time point, focal and cohabiting ants from each replicate were pooled
185 separately, flash-frozen in liquid nitrogen, and stored at -80 °C. RNA was extracted as
186 described above.
187

188 Focal and cohabiting ants sampled 24 h.p.i were used to assess viral replication via negative-
189 strand detection. This method relies on RT-qPCR amplification of negative-strand RNA
190 intermediates, which serve as markers of active replication of positive-strand RNA viruses
191 within host cells, rather than the presence of inert genomic RNA (de Miranda et al., 2010; Yue
192 & Genersch, 2005). Two-step RT-PCR was performed using a tagged forward primer
193 designed to specifically anneal to the complementary DNA (cDNA) product of the negative-
194 sense RNA strand of ABPV. Superscript III reverse transcriptase (Invitrogen, USA) was used
195 for cDNA synthesis. In preliminary tests, all ant and bee samples produced non-specific PCR
196 products using previously published primers (Schläppi et al., 2020). To avoid false positives,
197 we designed new primers for the assay. A total of 200 ng RNA was diluted to 10 µL with water
198 and combined with 1 µL of the tagged forward primer tag-ABPV(-)F5185 (5'-
199 AGCCTGCGCACCGTGG-TTGGTTTGGTGCA

200 GAAGGTG-3') and 1 µL 10 mM dNTPs. The mixture was incubated at 65 °C for 5 min and
201 then placed on ice for at least 1 min. Subsequently, 4 µL of 5X First-Strand Buffer, 1 µL of 0.1
202 M DTT, and 1 µL of Superscript III Reverse Transcriptase were added, bringing the total
203 reaction volume to 20 µL. The reaction was incubated at 55 °C for 50 min, followed by
204 incubation at 70 °C for 15 min to inactivate the enzyme. cDNA was purified using the
205 NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, Düren, Germany) and eluted in 20
206 µL of elution buffer. The purified cDNA (diluted 1:3 with nuclease-free water) served as the
207 template for PCR amplification with Taq polymerase (New England Biolabs), using a tag
208 oligonucleotide (5'-AGCCTGCGCACCGTGG-3') as the forward primer and the corresponding
209 reverse primer, ABPV(-)R5380 (5'-AGAAAAGTCCATAGGCCCGT-3'). PCR reactions without
210 the tag primer were performed for all samples to verify the complete removal of residual tagged
211 forward primers using the following cycling conditions: initial denaturation at 95 °C for 3 min;
212 35 cycles of denaturation at 95 °C for 20 s, annealing at 56 °C for 20 s, and extension at 68
213 °C for 30 s; and final extension at 68 °C for 2 min. The resulting PCR products were analysed
214 by electrophoresis on a 2% agarose gel. A 196 bp PCR product was expected in samples
215 containing negative-sense RNA, indicating active viral replication. Bee pupae injected with
216 control inoculum or ABPV inoculum served as negative and positive controls, respectively.
217

218 **(e) Replication Statement**

Scale of inference	Scale at which the factor of interest is applied	Number of replicates at the appropriate scale
Colony	Colony	4 control-injected colonies, 4 ABPV-injected colonies for all 4 time points

219

220 **(f) Statistical analyses**

221

222 All data analyses were performed using R version 4.4.1 (R Core Team, 2024). Models were
223 selected based on Akaike's Information Criterion (AIC) (Akaike, 1976) and compared using
224 the *anova* function to test if reduction of model terms significantly improved model fit. The final
225 model assumptions and residual diagnostics were assessed using the *DHARMa* package
226 (Hartig, 2020). Post-hoc pairwise comparisons were performed with the *emmeans* package
227 (Lenth et al., 2022) with Tukey's adjustment for multiple comparisons.
228

229 <u>Survival</u>

230 Due to complete separation in the pupal development data (no deaths in the control group and
231 no eclosion in the ABPV-inoculated group), Wilcoxon-Mann-Whitney tests were used to
232 compare the proportion of pupae that hatched into adults or died between treatment groups.
233 For adult survival, the data violated the proportional hazard assumption, and endpoint mortality
234 was therefore analysed using a generalised linear model (GLM) with a binomial error structure
235 and logit link, with Treatment as a fixed factor and Colony as a random factor.
236
237 Viral load
238 The viral load in focal and cohabiting ants was quantified by converting the Ct values to GEs
239 using a standard curve, followed by a $\log_{10}$ transformation. These transformed titres were
240 analysed using a linear model (*lm* function, base R) with one focal and one cohabiting sample
241 per colony. To assess how viral load changed over time in focal individuals, models
242 included Treatment (control-injected vs. ABPV-injected) and Time point (0, 3, and 5 d.p.i. for
243 pupae; 0, 1, 3, and 5 d.p.i. for adults), and their interaction (Treatment × Time point) as fixed
244 effects. To examine the viral load differences between focal and cohabiting individuals, a
245 separate model was fitted for overlapping time points between the two groups
246 including Individual type (focal vs. cohabiting) as a fixed factor, along with Treatment and Time
247 point. For pupae infection assay, comparisons between focal and cohabiting individuals were
248 performed only within the virus-inoculated treatment group as cohabiting adults were only
249 sampled in colonies of virus-inoculated pupae; thus, only Individual type and Time point were
250 included as fixed effects.
251

3. Results

ABPV-injected ant pupae had drastically reduced eclosion rates compared to control-injected pupae 5 d.p.i. (mean ± SD: 0 ± 0% in ABPV, 75 ± 10.2% in controls; Wilcoxon–Mann–Whitney test: $z = 2.477$, $p = 0.029$; Figure 1A). Similarly, ABPV-injected pupae had higher mortality compared to controls (mean ± SD: 31.3 ± 16.1% mortality in ABPV, 0 ± 0% mortality in controls; Wilcoxon–Mann–Whitney test; $z = -2.461$, p-value = 0.0286).

ABPV-injected adult ants had higher mortality than control-injected adults (mean ± SD: 47.5 ± 31% in ABPV; 15 ± 12.9% in controls; $\chi^2(1) = 4.628$, $p = 0.031$), a 5.1-fold increase in the odds of death, mostly occurring 1 d.p.i (Figure 1B). Although virus-injected focal pupae and adults showed increased mortality, no deaths were observed among the cohabiting adults.

Viral load differed between ABPV-injected and control-injected ant pupae (ANOVA: $F_{1,18} = 51.802$, $p < 0.001$) and across time points ($F_{2,18} = 5.207$, $p = 0.016$) (Figure 2A). As expected, ABPV-injected pupae had higher viral loads than control-injected pupae at all time points (Tukey's post-hoc; Control vs. ABPV at 0 d.p.i.: $t = -7.197$, $p < 0.0001$; 3 d.p.i.: $t = -5.726$, $p < .0001$; 5 d.p.i: $t = -3.500$, $p = 0.003$). In ABPV-injected pupae, viral load remained consistently high up to 5 d.p.i. (Tukey's post-hoc; 0 vs. 5 d.p.i.: $t = 1.224$, $p = 0.447$).

In adult ants, viral loads differed between control- and ABPV-injected groups (ANOVA: treatment: $F_{1,16} = 140.252$, $p < 0.001$), and this difference varied across time points (ANOVA; treatment × time point interaction: $F_{3,16} = 3.87$, $p = 0.030$, Figure 2B). This effect was driven by a sharp decline in viral load in ABPV-injected adults within the first day post-injection (Tukey's post-hoc; 0 vs. 1 d.p.i.: $t = 4.306$, $p = 0.003$), decreasing from approximately $10^{3.08}$ to $10^{1.42}$ GEs per individual. Thereafter, viral titres remained at a similar level up to day 5 (Tukey's post-hoc; 1 vs. 5 d.p.i.: $t = 0.795$, $p = 0.856$).

We consistently detected ABPV in uninjected adults that had cohabited with ABPV-injected pupae (Figure 2A) and adults (Figure 2B) for one to five days. Strikingly, adults cohabiting with ABPV-injected pupae carried viral loads as high as those of the injected pupae themselves (ANOVA; focal pupae vs. cohabiting adults, $F_{1, 13} = 1.922$, $p = 0.189$), suggesting efficient transmission from pupae to adults. In contrast, adults cohabiting with ABPV-injected adults had viral loads lower than those of the injected adults (Tukey's post-hoc; cohabiting vs. focal: $t = -5.381$, $p < 0.0001$), yet they still had significantly higher viral loads than controls (Tukey's post-hoc; control-cohabiting vs. ABPV-cohabiting: $t = -6.615$, $p < 0.0001$). This indicates rapid

287 and efficient horizontal transmission of the virus, both from the brood to adults and between
288 adults.
289
290 In line with the lack of increase in viral load over time, we found no evidence of viral replication
291 in adult ants. Viral negative-strand replication intermediates were consistently detected in
292 positive controls (ABPV-injected honey bee pupae), but not in ABPV-injected, control-injected,
293 and cohabiting adult ants 24 h post-injection (Figure 3).
294

295

4. Discussion

Our study shows that injection of ABPV into *O. biroi* pupae and adults reduced host fitness by delaying development and decreasing survival. ABPV persisted over days in inoculated adults and pupae and efficiently spread to adults. Strikingly, uninoculated nestmates acquired ABPV titres comparable to those of directly inoculated ants. These results demonstrate that ABPV particles can persist and spread in ant colonies, and can impact host fitness, even in the absence of detectable viral replication.

The mortality observed in ABPV-injected *O. biroi* ads to the growing evidence that honey bee viruses such as ABPV can impair fitness in non-honey bee hosts, with outcomes depending on the inoculation methods and dosage. For example, black garden ants (*Lasius niger*) fed on ABPV-infected bee pupae for ten weeks showed impaired locomotion and reduced adult emergence (Schläppi et al., 2020). Similarly, IAPV, a close relative of ABPV (de Miranda et al., 2010), reduces host reproductive success and survival when fed to bumblebees at very high doses (2×10^7 and 1×10^8 particles) (Meeus et al., 2014; Piot et al., 2015; Wang et al., 2018), while injections of lower doses of IAPV (ca. 10^2 particles) were sufficient to cause acute infection, paralysis, and mortality in bumblebee workers (Niu et al., 2014; Wang et al., 2016).

Despite strong host fitness impairment, we found no evidence of viral replication in *O. biroi* adults, as shown by stable or decreasing viral loads over time and a lack of detectable replicative viral strands. This suggests that fitness costs arise from host immunopathology (i.e., immune-mediated self-damage) rather than active infection (González-González & Wayne, 2020; Paludan et al., 2024), a common phenomenon across systems (Khan et al., 2017; Lazzaro & Tate, 2022; Rouse & Sehrawat, 2010; Scriba et al., 2024). For example, ABPV particles may trigger antiviral defences such as apoptosis and phagocytosis, but excessive apoptosis near vital organs can harm the host (Clarke & Clem, 2003; Nainu et al., 2017). Similarly, activation of the phenoloxidase cascade and the release of cytotoxic intermediates or reactive oxidative species confer broad antimicrobial activity, but can cause host tissue damage and early senescence (Dowling & Simmons, 2009; Mikonranta et al., 2014; Nappi & Christensen, 2005; Sadd & Siva-Jothy, 2006; Wu et al., 2012). In flies, overactivation or misregulation of the Imd pathway during early innate immune responses to invading pathogens or even benign gut microorganisms can lead to excessive immune responses that cause host mortality (Li et al., 2020; Paredes et al., 2011).

We speculate that the lack of ABPV replication in *O. biroi* is due to host specificity limiting viral infectivity and pathogenicity in a novel host (Doublet et al., 2025; Woolhouse et al., 2005).

Host phylogeny strongly predicts infection outcomes, as shown for the *Drosophila* C virus and the Cricket paralysis virus, where changes in viral load correlate with relatedness between natural and novel hosts (Imrie et al., 2021; Longdon et al., 2011). Successful infection typically requires compatible host cell surface receptors for viral attachment and entry (Maginnis et al., 2018; Weaver et al., 2021) and ABPV may lack the receptor-binding domains required to interact with *O. biroi* cells. Additionally, viral host shifts are often constrained by host-specific physiological and immune barriers, requiring substantially higher inoculation doses to overcome immunity and initiate replication in a novel host (Gusachenko et al., 2020; Schläppi et al., 2020; Streicher et al., 2023; Tehel et al., 2022), a threshold potentially not reached in our experiments. Taken together with cross-species infection experiments in ants (Schläppi et al., 2020; Valizadeh et al., 2025) and bumblebees (Gusachenko et al., 2020; Tehel et al., 2022; Wang et al., 2018), our results indicate that although honey bee viruses can spill over into novel hosts and impair fitness, onwards interspecific transmission or spillback to honey bees is not guaranteed and greatly depends on exposure dosage and host-virus compatibility (Rivas Fontan et al., 2025; Tiritelli et al., 2024).

Adult ants cohabiting with ABPV-injected pupae or adults acquired detectable viral loads within one to three days. Despite an *ad libitum* diet, some ABPV-inoculated pupae disappeared or were found partially consumed, indicating brood cannibalism by workers, a common behaviour in *O. biroi*. In termites (Sun et al., 2013) and ants (Bizzell & Pull, 2024; Maák et al., 2020), brood cannibalism serves both hygienic (removal of infectious material) and nutritional (resource recycling) functions. Thus, consumption of infected nestmates or their carcasses by workers represents a plausible and efficient viral uptake route, consistent with findings in honey bees, where viruses can remain infectious in host tissues for 48 hours post-mortem (Payne et al., 2025) and where worker cannibalism of infected pupae is a major source of DWV spread within colonies (Posada-Florez et al., 2021). Cannibalism would also explain the unexpectedly high viral loads in adults cohabiting with pupae in our experiment, if entire infected pupae were consumed. However, we cannot rule out that viral spread may also have occurred by social contact, such as mutual grooming and brood care, or via environmental contamination of the shared nest space (Dalmon et al., 2021; Tehel et al., 2022).

Our work adds to the evidence that even without sustaining viral replication, ants can acquire and harbour bee viruses for extended periods (Schläppi et al., 2019; Valizadeh et al., 2025), potentially serving as incidental hosts or mechanical reservoirs that facilitate viral persistence in insect communities (Dobelmann et al., 2024). Positive-sense RNA viruses, such as ABPV, replicate rapidly and with high error rates, which can accelerate their adaptation to novel hosts (de Miranda et al., 2010). Consequently, even transient uptake by ants may promote low-level

viral persistence that, under selective pressure, may give rise to variants capable of overcoming interspecific barriers and initiating host shifts, a process recognised as a key driver of emerging infectious diseases (Woolhouse et al., 2005). Environmental stressors, such as pesticide exposure (Al Naggar & Paxton, 2021; Schläppi et al., 2021) and climate change (Roberts et al., 2018), which affect host immune function and physiology, can potentially amplify the risk of adaptation to novel hosts and future spillover or spillback events, thereby shaping the epidemiology of bee pathogens in insect communities. Understanding these dynamics is critical for predicting viral emergence and designing strategies to safeguard pollinator health and enhance ecosystem resilience.

References

Akaike, H. (1976). Canonical correlation analysis of time series and the use of an information criterion. *Mathematics in Science and Engineering*, *126*, 27–96. https://doi.org/10.1016/S0076-5392(08)60869-3

Al Naggar, Y., & Paxton, R. J. (2021). The novel insecticides flupyradifurone and sulfoxaflor do not act synergistically with viral pathogens in reducing honey bee (Apis mellifera) survival but sulfoxaflor modulates host immunocompetence. *Microbial Biotechnology*, *14*(1), 227–240. https://doi.org/10.1111/1751-7915.13673

Azzami, K., Ritter, W., Tautz, J., & Beier, H. (2012). Infection of honey bees with acute bee paralysis virus does not trigger humoral or cellular immune responses. *Archives of Virology*, *157*(4), 689–702. https://doi.org/10.1007/s00705-012-1223-0

Bailey, L., & Milne, R. G. (1969). The multiplication regions and interaction of acute and chronic bee-paralysis viruses in adult honey bees. *The Journal of General Virology*, *4*(1), 9–14. https://doi.org/10.1099/0022-1317-4-1-9

Ball, B. V. (1985). Acute paralysis virus isolates from honeybee colonies infested withVarroa jacobsoni. *Journal of Apicultural Research*, *24*(2), 115–119. https://doi.org/10.1080/00218839.1985.11100658

Ball, B. V., & Allen, M. F. (1988). The prevalence of pathogens in honey bee (*Apis mellifera*) colonies infested with the parasitic mite *Varroa jacobsoni*. *The Annals of Applied Biology*, *113*(2), 237–244. https://doi.org/10.1111/j.1744-7348.1988.tb03300.x

Benjeddou, M., Leat, N., Allsopp, M., & Davison, S. (2001). Detection of acute bee paralysis virus and black queen cell virus from honeybees by reverse transcriptase pcr. *Applied and Environmental Microbiology*, *67*(5), 2384–2387. https://doi.org/10.1128/AEM.67.5.2384-2387.2001

Berthoud, H., Imdorf, A., Haueter, M., Radloff, S., & Neumann, P. (2010). Virus infections and winter losses of honey bee colonies (Apis mellifera). *Journal of Apicultural Research*, *49*(1), 60–65. https://doi.org/10.3896/ibra.1.49.1.08

Bizzell, F., & Pull, C. D. (2024). Ant queens cannibalise infected brood to contain disease spread and recycle nutrients. *Current Biology: CB*, *34*(18), R848–R849. https://doi.org/10.1016/j.cub.2024.07.062

Carreck, N. L., Ball, B. V., & Martin, S. J. (2010). Honey bee colony collapse and changes in viral prevalence associated with Varroa destructor. *Journal of Apicultural Research*, *49*(1), 93–94. https://doi.org/10.3896/ibra.1.49.1.13

Celle, O., Blanchard, P., Olivier, V., Schurr, F., Cougoule, N., Faucon, J.-P., & Ribière, M. (2008). Detection of Chronic bee paralysis virus (CBPV) genome and its replicative RNA form in various hosts and possible ways of spread. *Virus Research*, *133*(2), 280–284. https://doi.org/10.1016/j.virusres.2007.12.011

Clarke, T. E., & Clem, R. J. (2003). Insect defenses against virus infection: the role of apoptosis. *International Reviews of Immunology*, *22*(5–6), 401–424. https://doi.org/10.1080/08830180305215

Cox-Foster, D. L., Conlan, S., Holmes, E. C., Palacios, G., Evans, J. D., Moran, N. A., Quan, P.-L., Briese, T., Hornig, M., Geiser, D. M., Martinson, V., vanEngelsdorp, D., Kalkstein, A. L., Drysdale, A., Hui, J., Zhai, J., Cui, L., Hutchison, S. K., Simons, J. F., … Lipkin, W. I. (2007). A metagenomic survey of microbes in honey bee colony collapse disorder. *Science (New York, N.Y.)*, *318*(5848), 283–287. https://doi.org/10.1126/science.1146498

Dalmon, A., Diévart, V., Thomasson, M., Fouque, R., Vaissière, B. E., Guilbaud, L., Le Conte, Y., & Henry, M. (2021). Possible spillover of pathogens between bee communities foraging on the same floral resource. *Insects*, *12*(2), 122. https://doi.org/10.3390/insects12020122

de Miranda, J. R., Cordoni, G., & Budge, G. (2010). The Acute bee paralysis virus-Kashmir bee virus-Israeli acute paralysis virus complex. *Journal of Invertebrate Pathology*, *103 Suppl 1*, S30-47. https://doi.org/10.1016/j.jip.2009.06.014

Dobelmann, J., Felden, A., & Lester, P. J. (2023). An invasive ant increases deformed wing virus loads in honey bees. *Biology Letters*, *19*(1), 20220416. https://doi.org/10.1098/rsbl.2022.0416

Dobelmann, J., Manley, R., & Wilfert, L. (2024). Caught in the act: the invasion of a viral vector changes viral prevalence and titre in native honeybees and bumblebees. *Biology Letters*, *20*(5), 20230600. https://doi.org/10.1098/rsbl.2023.0600

Doublet, V., Doyle, T. D., Carvell, C., Brown, M. J. F., & Wilfert, L. (2025). Host ecology and phylogeny shape the temporal dynamics of social bee viromes. *Nature Communications*, *16*(1), 2207. https://doi.org/10.1038/s41467-025-57314-7

Dowling, D. K., & Simmons, L. W. (2009). Reactive oxygen species as universal constraints in life-history evolution. *Proceedings. Biological Sciences*, *276*(1663), 1737–1745. https://doi.org/10.1098/rspb.2008.1791

Genersch, E., & Aubert, M. (2010). Emerging and re-emerging viruses of the honey bee (Apis mellifera L.). *Veterinary Research*, *41*(6), 54. https://doi.org/10.1051/vetres/2010027

González-González, A., & Wayne, M. L. (2020). Immunopathology and immune homeostasis during viral infection in insects. *Advances in Virus Research*, *107*, 285–314. https://doi.org/10.1016/bs.aivir.2020.06.001

Gruber, M. A. M., Cooling, M., Baty, J. W., Buckley, K., Friedlander, A., Quinn, O., Russell, J. F. E. J., Sébastien, A., & Lester, P. J. (2017). Single-stranded RNA viruses infecting the invasive Argentine ant, Linepithema humile. *Scientific Reports*, *7*(1), 3304. https://doi.org/10.1038/s41598-017-03508-z

Gusachenko, O., Woodford, L., Balbirnie-Cumming, K., Ryabov, E., & Evans, D. J. (2020). Evidence for and against deformed wing virus spillover from honey bees to bumble bees: a reverse genetic analysis. *Scientific Reports*, *10*. https://doi.org/10.1038/s41598-020-73809-3

Hartig, F. (2020). DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. *R Package Version 0.3*.

Imrie, R. M., Roberts, K. E., & Longdon, B. (2021). Between virus correlations in the outcome of infection across host species: Evidence of virus by host species interactions. *Evolution Letters*, *5*(5), 472–483. https://doi.org/10.1002/evl3.247

Khan, I., Agashe, D., & Rolff, J. (2017). Early-life inflammation, immune response and ageing. *Proceedings. Biological Sciences*, *284*(1850), 20170125. https://doi.org/10.1098/rspb.2017.0125

Kronauer, D. J. (2009). Recent advances in army ant biology (Hymenoptera: Formicidae). *Myrmecological News*, *12*, 51–65. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=9ef5fea186cdc69f4ccc4d1c8a9a5a1f5cd3a62d

Kronauer, D. J. C., Pierce, N. E., & Keller, L. (2012). Asexual reproduction in introduced and native populations of the ant Cerapachys biroi. *Molecular Ecology*, *21*(21), 5221–5235. https://doi.org/10.1111/mec.12041

Lazzaro, B. P., & Tate, A. T. (2022). Balancing sensitivity, risk, and immunopathology in immune regulation. *Current Opinion in Insect Science*, *50*(100874), 100874. https://doi.org/10.1016/j.cois.2022.100874

Lenth, R. V., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2022). Emmeans: estimated marginal means. *AKA Least-Squares Means*.

Levitt, A. L., Singh, R., Cox-Foster, D. L., Rajotte, E., Hoover, K., Ostiguy, N., & Holmes, E. C. (2013). Cross-species transmission of honey bee viruses in associated arthropods. *Virus Research*, *176*(1–2), 232–240. https://doi.org/10.1016/j.virusres.2013.06.013

465 Li, R., Zhou, H., Jia, C., Jin, P., & Ma, F. (2020). Drosophila Myc restores immune homeostasis of Imd pathway via
466 activating miR-277 to inhibit imd/Tab2. *PLoS Genetics*, *16*(8), e1008989.
467 https://doi.org/10.1371/journal.pgen.1008989
468 Libbrecht, R., Oxley, P. R., Keller, L., & Kronauer, D. J. C. (2016). Robust DNA methylation in the clonal raider ant
469 brain. *Current Biology: CB*, *26*(3), 391–395. https://doi.org/10.1016/j.cub.2015.12.040
470 Lin, C.-Y., Lee, C.-C., Nai, Y., Hsu, H.-W., Lee, C., Tsuji, K., & Yang, C. (2020). Deformed wing virus in two
471 widespread invasive ants: Geographical distribution, prevalence, and phylogeny. *Viruses*, *12*.
472 https://doi.org/10.3390/v12111309
473 Lo, Lai Ka; Ashmita Baruah; Paxton, Robert; Ulrich, Yuko, 2025, "Experimental infections with a honeybee virus
474 induce fitness costs in ant colonies", https://doi.org/10.17617/3.DUUTOC, Edmond, V2
475 Longdon, B., Hadfield, J. D., Webster, C. L., Obbard, D. J., & Jiggins, F. M. (2011). Host phylogeny determines
476 viral persistence and replication in novel hosts. *PLoS Pathogens*, *7*(9), e1002260.
477 https://doi.org/10.1371/journal.ppat.1002260
478 Maák, I., Tóth, E., Lenda, M., Lőrinczi, G., Kiss, A., Juhász, O., Czechowski, W., & Torma, A. (2020). Behaviours
479 indicating cannibalistic necrophagy in ants are modulated by the perception of pathogen infection level.
480 *Scientific Reports*, *10*. https://doi.org/10.1038/s41598-020-74870-8
481 McMahon, D. P., Fürst, M. A., Caspar, J., Theodorou, P., Brown, M. J. F., & Paxton, R. J. (2015). A sting in the
482 spit: widespread cross-infection of multiple RNA viruses across wild and managed bees. *The Journal of*
483 *Animal Ecology*, *84*(3), 615–624. https://doi.org/10.1111/1365-2656.12345
484 McMenamin, A. J., & Genersch, E. (2015). Honey bee colony losses and associated viruses. *Current Opinion in*
485 *Insect Science*, *8*, 121–129. https://doi.org/10.1016/j.cois.2015.01.015
486 Meeus, I., de Miranda, J. R., de Graaf, D. C., Wäckers, F., & Smagghe, G. (2014). Effect of oral infection with
487 Kashmir bee virus and Israeli acute paralysis virus on bumblebee (Bombus terrestris) reproductive
488 success. *Journal of Invertebrate Pathology*, *121*, 64–69. https://doi.org/10.1016/j.jip.2014.06.011
489 Mikonranta, L., Mappes, J., Kaukoniitty, M., & Freitak, D. (2014). Insect immunity: oral exposure to a bacterial
490 pathogen elicits free radical response and protects from a recurring infection. *Frontiers in Zoology*, *11*(1),
491 23. https://doi.org/10.1186/1742-9994-11-23
492 Nainu, F., Shiratsuchi, A., & Nakanishi, Y. (2017). Induction of apoptosis and subsequent phagocytosis of virus-
493 infected cells as an antiviral mechanism. *Frontiers in Immunology*, *8*, 1220.
494 https://doi.org/10.3389/fimmu.2017.01220
495 Nappi, A. J., & Christensen, B. M. (2005). Melanogenesis and associated cytotoxic reactions: applications to insect
496 innate immunity. *Insect Biochemistry and Molecular Biology*, *35*(5), 443–459.
497 https://doi.org/10.1016/j.ibmb.2005.01.014
498 Neumann, P., & Carreck, N. L. (2010). Honey bee colony losses. *Journal of Apicultural Research*, *49*(1), 1–6.
499 https://doi.org/10.3896/ibra.1.49.1.01
500 Niu, J., Cappelle, K., de Miranda, J. R., Smagghe, G., & Meeus, I. (2014). Analysis of reference gene stability after
501 Israeli acute paralysis virus infection in bumblebees Bombus terrestris. *Journal of Invertebrate Pathology*,
502 *115*, 76–79. https://doi.org/10.1016/j.jip.2013.10.011
503 Paludan, S. R., Pradeu, T., Pichlmair, A., Wray, K. B., Mikkelsen, J. G., Olagnier, D., & Mogensen, T. H. (2024).
504 Early host defense against virus infections. *Cell Reports*, *43*(12), 115070.
505 https://doi.org/10.1016/j.celrep.2024.115070
506 Paredes, J. C., Welchman, D. P., Poidevin, M., & Lemaitre, B. (2011). Negative regulation by amidase PGRPs
507 shapes the Drosophila antibacterial response and protects the fly from innocuous infection. *Immunity*,
508 *35*(5), 770–779. https://doi.org/10.1016/j.immuni.2011.09.018

Parker, J., & Kronauer, D. J. C. (2021). How ants shape biodiversity. *Current Biology: CB*, *31*(19), R1208–R1214. https://doi.org/10.1016/j.cub.2021.08.015

Pascall, D. J., Tinsley, M. C., Clark, B. L., Obbard, D. J., & Wilfert, L. (2021). Virus prevalence and genetic diversity across a wild bumblebee community. *Frontiers in Microbiology*, *12*, 650747. https://doi.org/10.3389/fmicb.2021.650747

Payne, A. N., Prayugo, V., & Dolezal, A. G. (2025). A honey bee-associated virus remains infectious and quantifiable in postmortem hosts. *Journal of Invertebrate Pathology*, *209*(108258), 108258. https://doi.org/10.1016/j.jip.2024.108258

Payne, A. N., Shepherd, T. F., & Rangel, J. (2020). The detection of honey bee (Apis mellifera)-associated viruses in ants. *Scientific Reports*, *10*. https://doi.org/10.1038/s41598-020-59712-x

Piot, N., Snoeck, S., Vanlede, M., Smagghe, G., & Meeus, I. (2015). The effect of oral administration of dsRNA on viral replication and mortality in Bombus terrestris. *Viruses*, *7*(6), 3172–3185. https://doi.org/10.3390/v7062765

Posada-Florez, F., Lamas, Z. S., Hawthorne, D. J., Chen, Y., Evans, J. D., & Ryabov, E. V. (2021). Pupal cannibalism by worker honey bees contributes to the spread of deformed wing virus. *Scientific Reports*, *11*(1), 8989. https://doi.org/10.1038/s41598-021-88649-y

Ravary, F., Jahyny, B., & Jaisson, P. (2006). Brood stimulation controls the phasic reproductive cycle of the parthenogenetic ant Cerapachys biroi. *Insectes Sociaux*, *53*(1), 20–26. https://doi.org/10.1007/s00040-005-0828-7

Rivas Fontan, I., Gonzalez, F. N., Moja, J., Ferrufino, C., Zanola, D., Scally, B., Calcaterra, L., Dus Santos, M. J., & Josens, R. (2025). Assessing potential viral transmission between honeybees and hive-infesting ants using novel per-hive co-detection indices. *Apidologie*, *56*(4). https://doi.org/10.1007/s13592-025-01190-z

Roberts, K. E., Hadfield, J. D., Sharma, M. D., & Longdon, B. (2018). Changes in temperature alter the potential outcomes of virus host shifts. *PLoS Pathogens*, *14*(10), e1007185. https://doi.org/10.1371/journal.ppat.1007185

Rouse, B. T., & Sehrawat, S. (2010). Immunity and immunopathology to viruses: what decides the outcome? *Nature Reviews. Immunology*, *10*(7), 514–526. https://doi.org/10.1038/nri2802

Ryabov, E. V., Wood, G. R., Fannon, J. M., Moore, J. D., Bull, J. C., Chandler, D., Mead, A., Burroughs, N., & Evans, D. J. (2014). A virulent strain of deformed wing virus (DWV) of honeybees (Apis mellifera) prevails after Varroa destructor-mediated, or in vitro, transmission. *PLoS Pathogens*, *10*(6), e1004230. https://doi.org/10.1371/journal.ppat.1004230

Sadd, B. M., & Siva-Jothy, M. T. (2006). Self-harm caused by an insect's innate immunity. *Proceedings. Biological Sciences*, *273*(1600), 2571–2574. https://doi.org/10.1098/rspb.2006.3574

Schläppi, D., Chejanovsky, N., Yañez, O., & Neumann, P. (2020). Foodborne transmission and clinical symptoms of honey bee viruses in ants Lasius spp. *Viruses*, *12*. https://doi.org/10.3390/v12030321

Schläppi, D., Kettler, N., Glauser, G., Straub, L., Yañez, O., & Neumann, P. (2021). Varying impact of neonicotinoid insecticide and acute bee paralysis virus across castes and colonies of black garden ants, Lasius niger (Hymenoptera: Formicidae). *Scientific Reports*, *11*(1), 20500. https://doi.org/10.1038/s41598-021-98406-w

Schläppi, D., Lattrell, P., Yañez, O., Chejanovsky, N., & Neumann, P. (2019). Foodborne transmission of deformed wing virus to ants (Myrmica rubra). *Insects*, *10*. https://doi.org/10.3390/insects10110394

Schultheiss, P., Nooten, S. S., Wang, R., Wong, M. K. L., Brassard, F., & Guénard, B. (2022). The abundance, biomass, and distribution of ants on Earth. *Proceedings of the National Academy of Sciences of the United States of America*, *119*(40), e2201550119. https://doi.org/10.1073/pnas.2201550119

Scriba, T. J., Maseeme, M., Young, C., Taylor, L., & Leslie, A. J. (2024). Immunopathology in human tuberculosis. *Science Immunology*, *9*(102), eado5951. https://doi.org/10.1126/sciimmunol.ado5951

Sébastien, A., Lester, P. J., Hall, R. J., Wang, J., Moore, N. E., & Gruber, M. A. M. (2015). Invasive ants carry novel viruses in their new range and form reservoirs for a honeybee pathogen. *Biology Letters*, *11*(9), 20150610. https://doi.org/10.1098/rsbl.2015.0610

Streicher, T., Tehel, A., Tragust, S., & Paxton, R. J. (2023). Experimental viral spillover can harm *Bombus terrestris* workers under field conditions. *Ecological Entomology*, *48*(1), 81–89. https://doi.org/10.1111/een.13203

Sun, Q., Haynes, K. F., & Zhou, X. (2013). Differential undertaking response of a lower termite to congeneric and conspecific corpses. *Scientific Reports*, *3*(1), 1650. https://doi.org/10.1038/srep01650

Tehel, A., Streicher, T., Tragust, S., & Paxton, R. J. (2022). Experimental cross species transmission of a major viral pathogen in bees is predominantly from honeybees to bumblebees. *Proceedings. Biological Sciences*, *289*(1969), 20212255. https://doi.org/10.1098/rspb.2021.2255

Tiritelli, R., Cilia, G., & Grasso, D. A. (2024). Occurrence of honey bee (Apis mellifera) pathogens in ants (Hymenoptera: Formicidae): a systematic review. *Myrmecological News*, *34*, 91–104. https://doi.org/10.25849/MYRMECOL.NEWS_034:091

Tiritelli, R., Giannetti, D., Schifani, E., Grasso, D. A., & Cilia, G. (2025). Neighbors sharing pathogens: the intricate relationship between Apis mellifera and ants (Hymenoptera: Formicidae) nesting in hives. *Insect Science*, *32*(3), 943–956. https://doi.org/10.1111/1744-7917.13433

Valizadeh, B., Hardy, J., Chen, J., & Amiri, E. (2025). Dynamics of Israeli acute paralysis virus in the red imported fire ant (Solenopsis invicta Buren) colonies. *Journal of Invertebrate Pathology*, *211*(108310), 108310. https://doi.org/10.1016/j.jip.2025.108310

Wang, H., Meeus, I., Piot, N., & Smagghe, G. (2018). Systemic Israeli acute paralysis virus (IAPV) infection in bumblebees (Bombus terrestris) through feeding and injection. *Journal of Invertebrate Pathology*, *151*, 158–164. https://doi.org/10.1016/j.jip.2017.11.015

Wang, H., Meeus, I., & Smagghe, G. (2016). Israeli acute paralysis virus associated paralysis symptoms, viral tissue distribution and Dicer-2 induction in bumblebee workers (Bombus terrestris). *The Journal of General Virology*, *97*(8), 1981–1989. https://doi.org/10.1099/jgv.0.000516

Wiegers, F. P. (2021). Transmission of honeybee viruses by Varroa jacobsoni Oud. In *European Research on Varroatosis Control* (1st Edition, pp. 99–104). CRC Press. https://doi.org/10.1201/9781003211464-25

Woolhouse, M. E., Haydon, D. T., & Antia, R. (2005). Emerging pathogens: the epidemiology and evolution of species jumps. *Trends in Ecology & Evolution*, *20*(5), 238–244. https://www.cell.com/trends/ecology-evolution/fulltext/S0169-5347(05)00038-8?large_figure=true

Wu, S.-C., Liao, C.-W., Pan, R.-L., & Juang, J.-L. (2012). Infection-induced intestinal oxidative stress triggers organ-to-organ immunological communication in Drosophila. *Cell Host & Microbe*, *11*(4), 410–417. https://doi.org/10.1016/j.chom.2012.03.004

Yañez, O., Piot, N., Dalmon, A., de Miranda, J. R., Chantawannakul, P., Panziera, D., Amiri, E., Smagghe, G., Schroeder, D., & Chejanovsky, N. (2020). Bee viruses: Routes of infection in Hymenoptera. *Frontiers in Microbiology*, *11*, 943. https://doi.org/10.3389/fmicb.2020.00943

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics*, *13*(1), 134. https://doi.org/10.1186/1471-2105-13-134

Yue, C., & Genersch, E. (2005). RT-PCR analysis of Deformed wing virus in honeybees (Apis mellifera) and mites (Varroa destructor). *The Journal of General Virology*, *86*(Pt 12), 3419–3424. https://doi.org/10.1099/vir.0.81401-0

Žvokelj, L., Bakonyi, T., Korošec, T., & Gregorc, A. (2020). Appearance of acute bee paralysis virus, black queen cell virus and deformed wing virus in Carnolian honey bee (Apis mellifera carnica) queen rearing. *Journal of Apicultural Research*, *59*(1), 53–58. https://doi.org/10.1080/00218839.2019.1681115

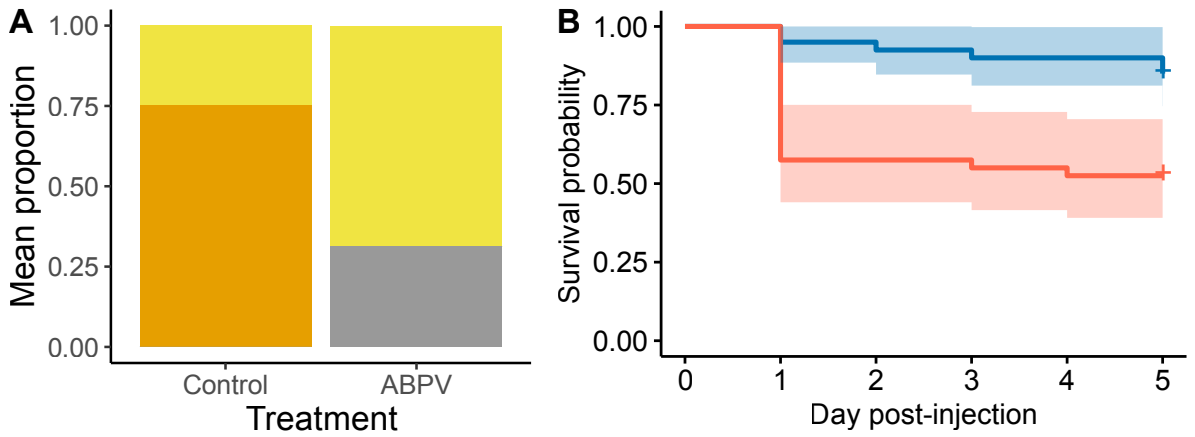

Figure 1. ABPV affects *O. biroi* development and survival. (A) Development and survival of ABPV-injected (10^5 GEs, n=4 colonies) and control-injected pupae (n=4 colonies), shown as the mean proportion of pupae (yellow) that eclosed into adults (orange) or died (gray) five days post-injection. **(B)** Survival of ABPV-injected (5×10^4 GEs, red, n=40 individuals) and control-injected (blue) adult ants (n=40 individuals). Kaplan–Meier curves with 95% confidence intervals.

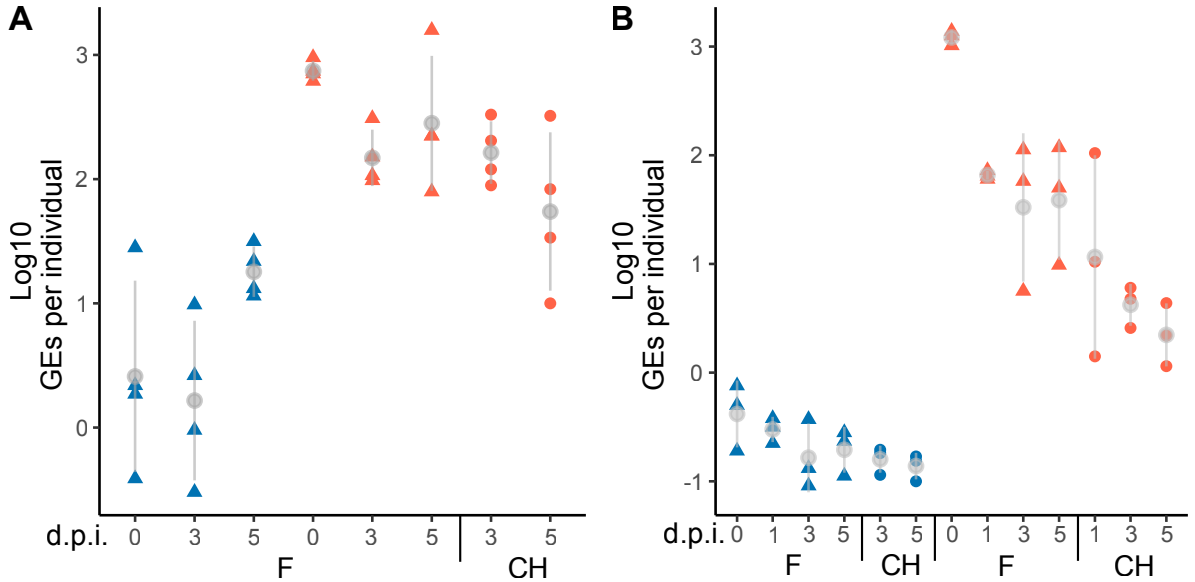

Figure 2. ABPV spreads within ant colonies. (A) Viral load in control-injected pupae (blue triangles), ABPV-injected pupae (red triangles), and cohabiting adults (red circles). Data points represent pools of three pupae or five cohabiting adults. Grey: mean ± SD. **(B)** Viral load in control-injected adults (blue triangles), ABPV-injected adults (red triangles), and cohabiting adults (blue and red circles). Data points represent pools of five adults (n = 3-4 per treatment and time point). d.p.i.: days post-injection. Grey: mean ± SD.

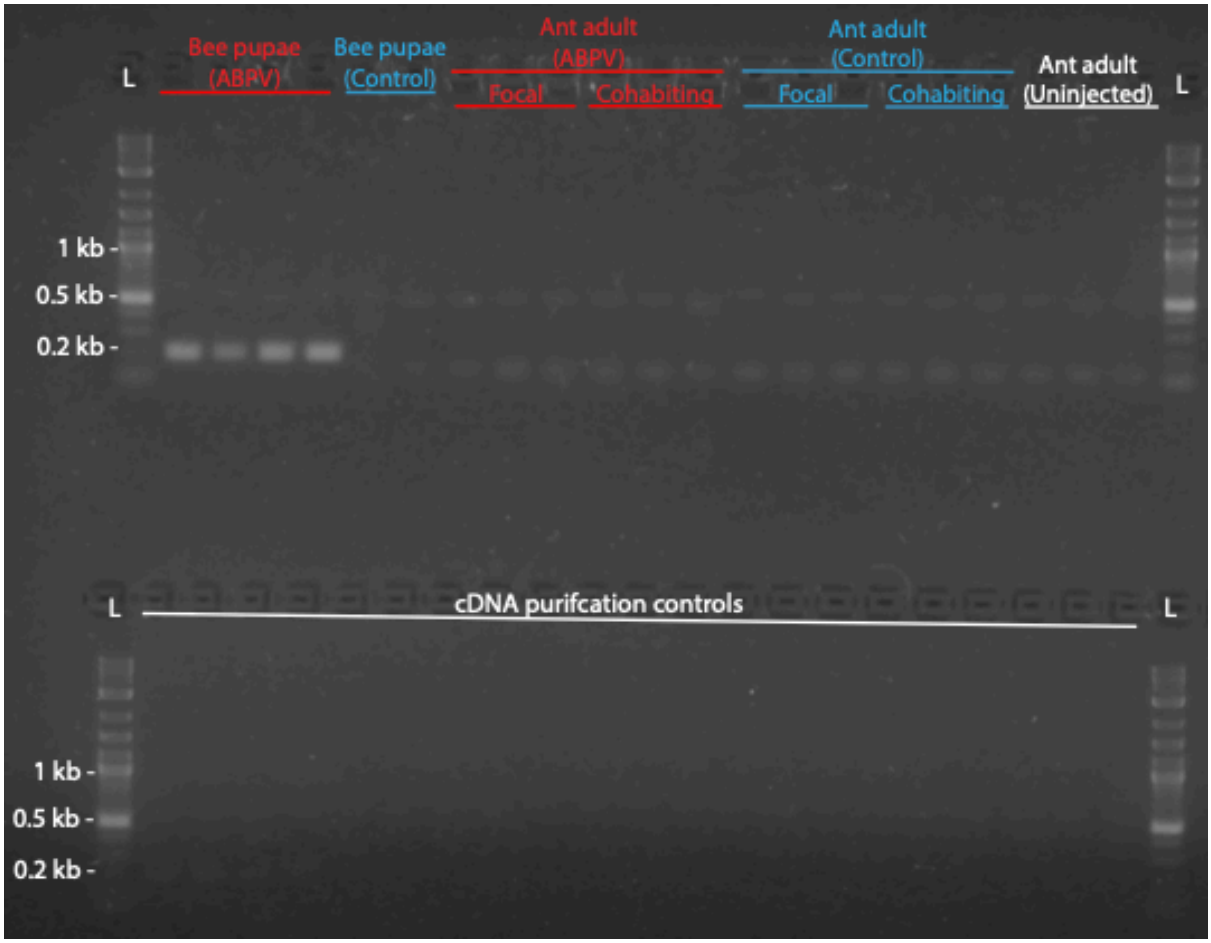

Figure 3. ABPV does not replicate in adult ants. Top row: ABPV-injected and control-injected bee pupae served as positive and negative controls, respectively. Red: ABPV-injected focals and cohabiting ants; blue: control-injected focals and cohabiting ants; white: uninjected. The expected PCR product size was 196 bp. L: DNA ladder. Bottom row: cDNA purification controls corresponding to the samples in the top row.